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High prevalence of multidrug-resistant *Pseudomonas aeruginosa* carrying integron and *exoA*, *exoS*, and *exoU* genes isolated from burn patients in Ahvaz, southwest Iran: A retrospective study

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Abstract

Background: *Pseudomonas aeruginosa* as an opportunistic pathogen produces several virulence factors. This study evaluated the relative frequency of exoenzymes (*exo*) A, U and S genes and integron classes (I, II, and III) among multi-drug-resistant clinical *P. aeruginosa* isolates from burn patients in Ahvaz, southwest of Iran.

Methods: In this cross-sectional study *P. aeruginosa* isolates were recovered from 355 wound samples. The antimicrobial susceptibility test was done by disk agar diffusion method on Muller-Hinton agar according to the Clinical and Laboratory Standards Institute. MDR isolates were defined if they showed simultaneous resistance to 3 antibiotics. Extensively drug-resistant was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories. The presence of class I, II, and III integrons and virulence genes was determined using a PCR assay on extracted DNA.

Results: Overall, 145 clinical *P. aeruginosa* isolates were confirmed with biochemical and PCR tests. Overall, 35% (52/145) of the isolates were taken from males and 64% (93/145) from female hospitalized burn patients. The highest resistance rates of *P. aeruginosa* isolates to antibiotics were related to piperacillin 59% (n = 86/145) and piperacillin-tazobactam 57% (n = 83/145). A total of 100% of isolates were resistant to at least one antibiotic. MDR and XDR *P. aeruginosa* had a frequency of 60% and 29%, respectively. The prevalence of integron classes I, II, and III in *P. aeruginosa* was 60%, 7.58%, and 3.44%, respectively. *IntI* was more common in MDR and XDR *P. aeruginosa* isolates. In addition, 70(48%) of *P. aeruginosa* isolates did not harbor integron genes. Besides, *exoA*, *exoS*, and *exoU* in *P. aeruginosa* had a frequency of 55%, 55%, and 56%, respectively.

Conclusion: It was found that *P. aeruginosa* as a potent pathogen with strong virulence factors and high antibiotic resistance in the health community can cause refractory diseases in burn patients.

KEYWORDS
antibiotic resistance, burn wound, exotoxin, integron, Pseudomonas aeruginosa

1 | INTRODUCTION

Pseudomonas aeruginosa is one of the most prevalent causes of healthcare-associated infections, including infections in the urinary system, respiratory tract, and surgical sites. This opportunistic organism is regarded as a severe health risk, particularly in immunocompromised people.[1,2] MDR has risen all throughout the world, posing a public health issue. Several recent studies have revealed the rise of MDR bacterial infections from various causes, emphasizing the need of correct antibiotic usage. Furthermore, routine antimicrobial susceptibility testing to identify the antibiotic of choice, as well as screening of new MDR strains, is crucial.[3–6]

P. aeruginosa possesses a virulence repertoire that significantly contributes to its pathogenicity. *P. aeruginosa* maintains both cell-mediated and secreted virulence factors. Cell-mediated virulence factors such as lipopolysaccharide (LPS), flagella, and pili are essential for bacterial movement, colonization of host tissues, and invasion of bacterial active proteins into target cells. Furthermore, secreted virulence types promote microbial invasion and multiplication, exacerbate inflammatory conditions, cause significant host-tissue damage, and raise infection severity. Exotoxin A and exotoxin S are the most prevalent virulence determinants released by *P. aeruginosa*. Exotoxin A prevents protein synthesis in the host cell, whereas exotoxin S is an external protein that causes cell death by activating GTPases and ribosyl transferases. Furthermore, the pathogen secretes physiologically active phenazine chemicals, which play an important role in bacterial pathogenicity.[7,8] Moreover, *P. aeruginosa* has an exceptional ability to produce biofilms, which makes it resistant to antimicrobials.[9]

Also, antibiotic resistance is spread through exchangeable genetic elements such as plasmids, transposons, and integrons in many bacteria. Integrons are frequent gene capture and expression systems that are made up of the promoter, attachment site (att I), and integrase gene (int I). Class 1 integrons, which carry single or multiple gene cassettes that give resistance to aminoglycosides, β-lactams, chloramphenicol, and macrolides, have been reported to be the most common in clinical isolates.[10,11] Despite the fact that class 2 integrons are coupled with a mobile DNA element, the Tn7 transposon, they are not as common in bacteria.[12] The prevalence of integrons varies by region in the world. For example, studies in Malaysia, China, and Iran found that 63%, 38%, and 35.6% of isolated *P. aeruginosa* have the class 1 integron gene, respectively.[13–15]

The identification of virulence genes' profile is crucial for developing efficient policies against *P. aeruginosa* infections; this study aimed to evaluate the distribution *of exoA*, *exoU*, and *exoS* genes and integron classes (I. II. III) among MDR and XDR *P. aeruginosa* isolated from burn patients in Ahvaz.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

The research obtained the approval of Research Ethics Committee (REC), Ahvaz Jundishapur University of Medical Sciences (No: IR.AJUMS.REC.1399.957). Based on Declaration of Helsinki, 1975, this observational study was without any new interventions.

2.2 | Specimen collection

The retrospective research was conducted on burn patients referring to Taleghani Hospital's Burn Center as the largest burn center (10 bed–ICU and 17 bed–ward) in Ahvaz, between May 2022 and November 2023. These samples were gathered from hospitalized patients of all genders and ages (16–90 years). Clinical samples were taken from infected burn sites while dressings were changed using cotton disposable swabs and conveyed to a sterile medium in plastic bottles. A protective gown and disposable gloves were utilized when coming into close touch with patients. The specimens were carried in a sterile, leak-proof container to the medical faculty's microbiology lab for diagnostic tests.

2.3 | Isolation and identification

Samples from the burn wounds were collected from 355 patients and were cultured in thyoglycollate medium (Merck KGaA), and subcultured on nutrient agar (Merck KGaA), MacConkey agar (MirMedia), and eosin-methylene blue (EMB) agar plate (MirMedia) by streaking method, and incubated for 24–48 h at 37°C. The isolates were confirmed to the species level by Gram staining to help identify growing colonies, and biochemical tests that included oxidase, catalase, sulfide indole motility[15] (Merck KGaA), methyl red-voges proskauer (MR-VP) (Quelabm), triple sugar iron (TSI) agar, simon's

citrate agar, nitrate broth, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, oxidation/fermentation of glucose, growth at 42°C, as well as gelatinase and pyocyanin production, hemolysis on blood agar, and the smell in cultures, according to standard microbiological methods. Afterward, Gram stain isolates were diagnosed by VITEK® 2 Compact Automated Systems with ID-GN and ID- Gp cards based on the manufacturer instructions. A total of 145 *P. aeruginosa* isolates were phenotypically identified by routine cultural and biochemical methods.

The boiling method was used to extract genomic DNA from *P. aeruginosa* isolates.[16] A few bacterial colonies of *P. aeruginosa* strains grown overnight on nutrient agar (Merck) were suspended in microtubes containing 500 µL of Tris-EDTA buffer. The microtubes were placed in cub lock microtube incubators (Denville Scientific) for 5 min at 95°C, and then centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was used as the DNA template in the PCR assays. The quality and average DNA yield were assessed using Nano Drop Spectrophotometer PROMO (Thermo Scientific).[17]

Amplification reactions were set up as detailed by Mohammed et al.[18] The following *16 S rRNA* specific primer set was used (Sigma-aldrich): 16 S forward primer: 5'-AGAGTRTGATCMTYGCTWAC-3'; 16S reverse primer: 5'-CGYTAMCTTWTTACGRCT-3'. Following optimization, reaction mixes (100 µL) were set up as follows: 10 mM Tris/HCl, pH 8·3; 50 mM KCl; 2·5 mM MgCl2; 200 µM (each) dATP, dCTP, dGTP and dTTP; 1·25U Taq DNA polymerase (Genei Bangalore); 0·1 µM (each) primer; and 4 µL DNA template. Reaction mixtures, following a "hot start," were subjected to the following empirically optimized thermal cycling parameters: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, followed by a final extension at 72°C for 5 min. Positive (*P. aeruginosa* ATCC 27853 DNA) and multiple negative control (water) were included in every set of PCRs. PCR products were run on 1.5% agarose gel and were afterward visualized under UV lamb.

2.4 | Drug susceptibility testing[12]

The antimicrobial susceptibility test was done by disk agar diffusion method on Muller-Hinton agar (Merck Co.). The media used is Mueller-Hinton agar at only 4 mm deep, which was poured into either 100 or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4. The bacterial inoculum is prepared by diluting a broth culture to match a 0.5 McFarland turbidity standard, which is equivalent to approximately 150 million cells per mL. Diffusion from a disc containing a defined dose of the antimicrobial agent placed on a plate seeded with bacteria produces a zone of inhibition. The edge of that zone occurs when the concentration of antimicrobial agent is inadequate to inhibit bacterial growth. Using CLSI recommendations, the width of the zone determines whether a strain is sensitive, intermediate, or resistant to the medication. As explained, this approach only produces categorical interpretive findings. Because solid medium tests are simple and inexpensive, they are widely employed. The following antibiotics were tested: 100 µg Piperacillin, 100/10 µg Piperacillin-tazobactam, 30/20 µg Ceftazidime-avibactam, 30/10 µg Ceftolozane-tazobactam, 75/10 µg Ticarcillin-clavulanate, 30 µg Ceftazidime, 30 µg Aztreonam, 10 µg Doripenem, 10 µg Imipenem, 30 µg Meropenem, 10 µg Gentamicin, 10 µg Tobramycin, 30 µg Amikacin, 30 µg Netilmicin, 5 µg Ciprofloxacin, 5 µg Levofloxacin, 10 µg Norfloxacin, 5 µg Ofloxacin, 5 µg Gatifloxacin (MAST Co.), in accordance with CLSI recommendations.[19] MDR isolates were defined if they showed simultaneous resistance to 3 antibiotics. Extensively drug resistant[10] was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories. The *P. aeruginosa* ATCC 27853 was used as a reference strain for the quality control of susceptibility test. According to Magiorakos et al.,[20] MDR and XDR definition is: The MDR isolate is non-susceptible to at least 1 agent in ≥3 antimicrobial categories listed in Table 1. The XDR isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1.

2.5 | Minimum inhibitory concentration (MIC)

Colistin MICs was determined using the described Vitek 2 system (bioMérieux) and interpreted by the aforesaid system as per CLSI guidelines.[19] After measuring the MIC with Vitek 2 system, the *P. aeruginosa* isolates with MIC values equal to or lower than 2 µg/mL were considered as susceptible and MIC values equal to or greater than 4 µg/mL were considered as resistant. *P. aeruginosa* ATCC 27853 was used as quality control for antimicrobial susceptibility testing.

2.6 | Multiple antibiotic resistance (MAR) indexing

P. aeruginosa was regarded as MAR if any showed resistance to two or more antimicrobial drugs. MAR index values were calculated following the procedure illustrated by Krumperman et al.[25] MAR index for a single isolate was calculated as the following formula: "Number of antibiotics to which isolate is resistant (a)/Total number of antibiotics against which isolate was tested (b)".

2.7 | Detection of class I, II, and III integrons and major virulence genes

Virulence-related genes (*exoA*, *exoU*, and *exoS*) and integron-related genes (class I, II, and III integrons) were detected using PCR. Bacterial DNA extraction was performed in accordance with the boiling method.[16] The PCR assay was carried out in a final volume of 25 µL containing Taq DNA polymerase (1 U; CinnaGen), dNTPs (100 µM), Taq buffer (5×), DNA template (50 ng), and forward and reverse primers (25 pM). PCR mixtures were subjected to the following thermal cycling: 5 min at 94°C, followed by 35 cycles with denaturation at 94°C for 50 s, annealing at 47–68°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Primer sequences used for the detection of the above-mentioned genes are presented in Table 2. The PCR amplicons were analyzed by

TABLE 1 Results of virulence factor, antibiotic resistance and integron in *Pseudomonas aeruginosa* isolates.

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
1	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	+	+	-	-	-	+	-	+
2	M	PIP, TZP, CZA, CAZ, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, LVX, GAT	+	+	-	+	-	-	+	-	+
3	F	PIP. CAZ, ATM, IMP, CIP, OFX, GEN, LVX, GAT, COL	+	+	-	-	+	-	+	+	-
4	F	PIP, TC, DOR, TOB, AMK, NET, LVX,, CIP, NOR	+	+	+	-	-	-	+	+	-
5	F	PIP, TZP, CT, CAZ, TC, ATM, DOR, GEN, CIP, AMK, LVX, NOR, OFX, COL	-	+	+	+	-	+	+	+	-
6	M	PIP, TZP, CT, CAZ, ATM, DOR, TOB, CIP, AMK, LVX, NOR, OFX, CIP	-	+	-	-	-	-	+	+	-
7	F	PIP, CZA, TC, CAZ, ATM, IMP, MEM, DOR, GEN, NET, CIP, OFX	+	+	+	-	+	-	+	+	-
8	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	+	-	-	+	-	+
9	M	TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, LVX, COL	-	+	-	-	-	-	+	+	-
10	F	TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, LVX	-	+	-	-	-	-	+	+	-
11	F	PIP, CZA, ATM, IMP, MEM, CIP, GEN, TOB, AMK, NET, LVX, NOR, COL	-	-	+	+	-	-	+	+	-
12	M	PIP, TZP, CZA, CAZ, ATM, DOR, IMP, MEM, CIP, GEN, TOB, AMK, LVX	+	-	+	-	-	-	+	+	-
13	F	CZA, TC, CAZ, ATM, DOR, IMP, AMK, CIP, NOR, TOB, AMK, OFX	-	+	+	-	-	-	+	+	-
14	F	PIP, CT, ATM, DOR, IMP, MEM, GEN, TOB, NET, OFX, NOR, COL	-	+	+	-	+	-	+	+	-
15	M	TC, CAZ, ATM, DOR, GEN, AMK, CIP, OFX, NOR	-	-	+	+	-	-	+	+	-
16	F	PIP, TZP, CAZ, DOR, IMP, MEM, CIP, TOB, NET, LVX, NOR, COL	-	+	+	-	-	-	+	+	-
17	F	TC, CAZ, ATM, DOR, IMP, GEN, AMK, CIP, OFX, NOR	+	+	+	+	-	-	+	+	-
18	M	PIP, CAZ, CT, ATM, IMP, MEM, TOB, CIP, NOR, COL	+	+	+	-	-	-	+	+	-
19	F	TC, CAZ, ATM, DOR, IMP, GEN, AMK, CIP, OFX, NOR	-	-	+	+	-	-	+	+	-
20	F	PIP, TZP, ATM, DOR, GEN, TOB, CIP, TOB, NET, LVX, NOR	+	-	+	-	+	-	+	+	-
21	M	CAZ, ATM, IMP, GEN, TOB, CIP, OFX, NOR, LVX	+	+	+	+	-	-	+	+	-
22	F	PIP, CT, CAZ, IMP, MEM, GEN, TOB, NET, LVX, NOR	+	+	+	-	-	-	+	+	-

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
23	F	CAZ, CAZ, ATM, DOR, IMP, GEN, NET, CIP, NOR	+	+	+	+	-	-	+	+	-
24	M	PIP, TZP, CT, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, COL	+	+	+	-	-	-	+	-	+
25	F	TZP, CT, ATM, IMP, CIP, GEN, TOB, AMK, NET, LVX	-	-	+	-	-	-	+	+	-
26	M	PIP, TZP, CT, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR	+	-	+	-	-	-	+	+	-
27	F	PIP, CZA, TC, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX	+	-	-	+	-	-	+	+	-
28	M	PIP, CZA, TC, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX	+	+	+	-	-	-	+	+	-
29	F	PIP, CZA, TC, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX, COL	-	+	+	+	-	-	+	+	-
30	F	CAZ, CAZ, ATM, DOR, IMP, GEN, NET, CIP, NOR	+	+	+	-	-	-	+	+	-
31	M	CAZ, CAZ, ATM, DOR, IMP, GEN, NET, CIP, NOR	+	+	-	+	-	-	+	+	-
32	F	CAZ, CAZ, ATM, DOR, IMP, GEN, NET, CIP, NOR, COL	+	+	-	-	-	-	+	+	-
33	F	PIP, TZP, CZA, TC, ATM, IMP, GEN, OFX, NET, CIP, COL	-	+	+	-	-	-	+	+	-
34	M	PIP, CZA, TC, ATM, IMP, GEN, TOB, AMK, NET, CIP	+	+	+	+	-	-	+	+	-
35	F	PIP, TZP, CT, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR	+	+	-	-	-	+	+	-	+
36	F	PIP, TZP, CT, CAZ, TC, ATM, DOR, IMP, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, COL	+	+	-	-	-	-	+	-	+
37	M	PIP, TZP, ATM, DOR, GEN, TOB, CIP, TOB, NET, LVX, NOR	+	+	+	+	-	-	+	+	-
38	F	CZA, CT, CAZ, ATM, DOR, IMP, AMK, LUX, NOR, COL	-	+	-	-	-	-	+	+	-
39	F	PIP, TZP, CZA, TC, CAZ, ATM, IMP, CIP, OFX, NOR	+	+	+	+	-	-	+	+	-
40	M	CZA, CT, CAZ, ATM, DOR, IMP, AMK, LUX, NOR, COL	+	+	+	-	-	+	+	+	-
41	F	CZA, CT, CAZ, ATM, DOR, IMP, AMK, LUX, NOR	+	+	-	-	-	-	+	+	-
42	F	CZA, CT, CAZ, ATM, DOR, IMP, AMK, LUX, NOR	-	+	-	+	-	-	+	+	-
43	M	PIP, TZP, ATM, DOR, GEN, TOB, CIP, TOB, NET, LVX, NOR	+	+	+	-	-	-	+	+	-

(Continues)

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
44	F	PIP, TZP, CT, TC, CAZ, ATM, DOR, CIP, TOB, NOR	-	-	-	-	-	-	+	+	-
45	M	TOB, AMK, CIP, LVX, NOR, OFX	+	-	+	+	-	-	+	+	-
46	F	PIP, TZP, CAZ, ATM, DOR, IMP, GEN, TOB, CIP	-	-	+	-	-	-	+	+	-
47	F	PIP, CAZ, GEN, ATM, DOR, IMP, GEN, TOB, CIP	-	-	+	+	-	-	+	+	-
48	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	+	-	-	-	-	+		+
49	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	-	+	-	-	-	+	-	+
50	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	+	+	-	-	-	+	-	+
51	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	-	-	+	-	-	+	-	+
52	F	PIP, TZP, CZA, CT	+	-	-	-	-	+	+	-	-
53	F	TZP, CZA, ATM, IMP, TOB, AMK, CIP, LVX, NOR, COL	+	-	-	-	-	-	+	+	-
54	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	+	+	-	-	+	-	+
55	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	-	-	-	-	-	+	+	-
56	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	-	+	-	-	-	-	+	+	-
57	M	CAZ, CT, TC, CAZ, MEM, CIP,, NET, LV, NOR	-	-	-	-	-	-	+	+	-
58	F	TZP, IMP, MEM, GEN, TOB, AMK, NET, LVX, NOR, COL	+	+	+	-	-	-	+	+	-
59	F	TZP, CZA, ATM, IMP, TOB, AMK, CIP, LVX, NOR	-	+	-	+	-	-	+	+	-
60	M	TZP, ATM, MEM, CIP, OFX, GEN, TOB, AMK, NET, NOR, COL	-	-	+	-	-	-	+	+	-
61	F	PIP, CZA, CAZ, GEN, LVX, NOR, OFX,	-	+	-	-	-	-	+	+	-
62	F	PIP, CZA, TC, CAZ, DOR, MEM, GEN, TOB, AMK, NOR, COL	-	+	+	-	-	-	+	+	-
63	M	PIP, CZA, ATM, MEM, GEN, TOB, AMK, NOR, LVX, NET, OFX	+	+	+	-	-	-	+	+	-
64	F	PIP, CZA, CAZ, GEN, TOB, AMK, NOR, CIP, OFX	+	-	+	+	-	-	+	+	-
65	F	PIP, CZA, CAZ, ATM, MEM, DOR, GEN, TOB, NOR, OFX	+	+	-	-	-	-	+	+	-

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
66	M	TZP, CZA, ATM, IMP, TOB, AMK, CIP, LVX, NOR	+	-	+	-	-	-	+	+	-
67	F	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR, COL	-	+	+	-	-	-	+	+	-
68	F	TZP, CZA, TC, ATM, DOR, MEM, TOB, CIP, AMK, NOR	+	+	+	-	-	-	+	+	-
69	F	CAZ, ATM, DOR, NET, LVX, NOR	+	-	-	-	-	-	+	+	-
70	M	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	+	+	-	-	-	+	+	-
71	F	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	-	-	+	+	-	-	+	+	-
72	F	PIP, CZA, CAZ, ATM, MEM, DOR, GEN, TOB, NOR, OFX, COL	-	+	-	-	-	-	+	+	-
73	M	PIP, CZA, CAZ, ATM, MEM, DOR, GEN, TOB, NOR, OFX	-	-	+	+	-	-	+	+	-
74	F	PIP, CZA, CAZ, ATM, MEM, DOR, GEN, TOB, NOR, OFX, COL	-	+	+	-	-	-	+	+	-
75	F	PIP, CZA, CAZ, ATM, MEM, DOR, GEN, TOB, NOR, OFX	-	+	-	+	-	-	+	+	-
76	M	PIP, TZP, CZA, CAZ, TC, ATM, DOR, MEM, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	+	-	-	-	-	+	+	-
77	F	TZP, ATM, DOR, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR	+	-	+	+	-	-	+	+	-
78	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, TOB, AMK	+	-	+	-	-	-	+	+	-
79	M	PIP, TC, ATM, MEM, TOB, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX	-	+	-	+	-	-	+	+	-
80	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, TOB, AMK	-	+	+	-	-	-	+	+	-
81	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, CIP, OFX, GEN, TOB, AMK	+	+	-	+	-	-	+	+	-
82	M	PIP, TC, ATM, MEM, TOB, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX	-	-	-	-	-	-	+	+	-
83	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, GEN, AMK, COL	-	+	+	+	-	-	+	+	-
84	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, TOB, AMK	+	+	-	-	-	-	+	+	-
85	M	PIP, TC, ATM, MEM, TOB, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX	+	+	+	+	-	-	+	+	-
86	F	CAZ, ATM, DOR, NET, LVX, NOR	+	-	+	-	-	-	+	+	-
87	M	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	-	-	+	+	-	-	+	+	-
88	F	CAZ, ATM, DOR, NET, LVX, NOR	-	+	-	-	-	-	+	+	-
89	M	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	-	-	-	+	-	-	+	+	-

(Continues)

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
90	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, GEN, AMK	-	+	-	-	-	-	+	+	-
91	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, GEN,	-	+	-	-	+	-	+	+	-
92	F	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	-	-	+	-	-	-	+	+	-
93	M	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	-	-	+	-	-	-	+	+	-
94	F	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	+	-	+	-	+	-	+	+	-
95	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX,	-	+	+	-	-	-	+	+	-
96	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	-	+	+	-	-	+	+	-
97	M	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	+	+	-	-	-	-	+	+	-
98	F	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	+	+	-	-	-	+	+	-
99	F	TZP, CZA, CAZ, CIP, IMP, MEM, GEN, CIP, NET, LVX, NOR	+	+	+	+	-	-	+	+	-
100	M	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	+	+	-	-	-	+	+	-
101	F	PIP, TZP, CZA, CT, CAZ	-	+	+	-	-	-	+	-	-
102	M	PIP, TZP, CZA, CT, CAZ	+	-	-	+	-	-	+	-	-
103	F	PIP, TZP, CZA, CT	-	+	+	+	-	-	+	-	-
104	M	CAZ, ATM, DOR, NET, LVX, NOR	+	-	+	-	-	-	+	-	+
105	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	-	-	+	-	-	+	-	+
106	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, GEN, TOB, AMK	+	-	-	-	-	-	+	-	+
107	M	CAZ, ATM, DOR, NET, LVX, NOR	+	+	-	+	-	-	+	-	+
108	F	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	+	+	+	+	-	-	+	-	+
109	M	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	-	+	+	-	-	+	-	+
110	M	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	+	+	+	-	+	-	+	-	+
111	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	+	-	-	+	-	+
112	M	PIP, CT, IMP, CIP, NOR, OFX, LVX, COL	+	+	+	+	-	-	+	-	+
113	F	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	+	-	-	+	-	-	+	-	+
114	F	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	-	+	-	-	-	-	+	-	+
116	F	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	-	+	+	-	-	+	-	+
117	F	CAZ, ATM, IMP, NET, CIP, AMK, CIP, LVX, NOR	+	-	-	-	-	-	+	-	+

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
118	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	-	+	+	-	-	+	-	+
119	F	CAZ, ATM, DOR, NET, LVX, NOR	+	-	-	-	-	-	+	-	+
120	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	-	-	+	-	-	+	-	+
121	M	PIP, CT, IMP, CIP, NOR, OFX, LVX	+	-	+	-	-	+	+	-	+
122	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	-	-	+	-	-	+	-	+
123	F	PIP, TZP, CZA, CT, CAZ	+	-	-	-	+	-	+	-	-
124	M	PIP, CT, IMP, CIP, NOR, OFX, LVX, COL	-	-	+	+	-	-	+	-	+
125	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	-	-	-	+	-	+
126	M	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	-	-	+	-	+	-	+	-	+
127	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	+	+	-	+	-	-	+	-	+
128	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, MEM, CIP, OFX, GEN, TOB, AMK, COL	+	-	+	-	+	-	+	-	+
129	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	+	+	-	-	-	-	+	-	+
130	F	PIP, TZP, CZA, CT, CAZ	-	-	-	+	-	-	+	-	-
131	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	-	+	-	+	-	+
132	F	PIP, TZP, CZA, CT, CAZ	-	-	-	-	-	-	+	-	-
133	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	+	-	-	+	-	+
134	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	+	+	-	-	+	-	+	-	+
135	M	PIP, TZP, CZA, CT, CAZ	+	+	+	-	-	-	+	-	-
136	F	PIP, TZP, CZA, CT, CAZ, TC	-	+	+	+	-	-	+	-	-
137	F	PIP, TZP, CZA, CT, CAZ, TC	+	-	-	-	-	-	+	-	-
138	F	PIP, TZP, CZA, CT, CAZ	+	-	+	+	-	-	+	-	-
139	M	PIP, TZP, CZA	+	+	-	-	-	+	+	-	-
140	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	+	-	+	-	-	-	+	-	+
141	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	-	+	-	+	-	-	+	-	+
142	F	PIP, TZP, CZA, CT, CAZ, TC, ATM, DOR, IMP, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT, COL	-	+	+	+	-	-	+	-	+
143	F	PIP, TZP, CZA, CT, CAZ	-	-	-	+	-	-	+	-	-

(Continues)

TABLE 1 (Continued)

ID	Sex	Antibiotics	Antimicrobial resistance genes						Type of resistance		
			exoA	exoS	exoU	I	II	III	R	MDR	XDR
144	F	PIP, TZP, CZA, CT	+	+	-	-	-	-	+	-	-
145	F	TZP, CZA, MEM, CIP, OFX, GEN, TOB, AMK, NET, LVX, NOR, GAT	-	+	-	+	-	-	+	-	+

Abbreviations: AMK, Amikacin; ATM, Aztreonam; CAZ, Ceftazidime; CIP, Ciprofloxacin; COL, Colistin; CT, Ceftolozane-Tazobactam; CZA, Ceftazidime-Avibactam; DOR, Doripenem; GAT, Gatifloxacin; GEN, Gentamicin; IPM, Imipenem; LVX, Levofloxacin; MEM, Meropenem; NET, Netilmicin; NOR, Norfloxacin; OFX, Ofloxacin; PIP, Piperacillin; TC, Ticarcillin-Clavulanic acid; TOB, Tobramycin; TZP, Piperacillin-Tazobactam.

TABLE 2 Primer sequences.

Gene	Primer sequence (5 '- 3')	Product size[15]	Annealing temperature	Reference
16 S rRNA	F- AGAGTRTGATCMTYGCTWAC R- CGYTAMCTTWTTACGRCT	1500	52	[18]
exoU	F-GGCACATATCTCCGGTTCCTTC R-TCAACTCAGCTGCCAACCATGC	761	55	[26]
exoS	F-ATGGCGTGTTCCGAGTCA R-AGGTGTCGGTTCGTGACGTCT	1587	55	[26]
exoA	F-AACCAGCTCAGCCACATGTC R-CGCTGGCCCATTCGCTCCAGCGCT	396	68	[22]
intI1	F-GGTGTGGCGGGCTTCGTG R-GCATCCTCGGTTTTCTGG	480	50	[27]
intI2	F- CTAGAATAGGCTGTATAGGCAGA R-GAGTGACGAAATGTATGACAAG	850	47	[27]
intI3	F-CAGTCTTTCCTCAAACAAGTG R-TACATCCTACAGACCGAGAAA	702	52	[27]

electrophoresis on 1.5% agarose gel in 1× Tris-Borate EDTA (TBE) buffer. The genome of *Escherichia coli* ATCC 35218 was used as the negative control.

2.8 | Data analysis

Descriptive data were analyzed using Microsoft Excel and SPSS version 22 statistics software (IBM Corporation). Cramer's V correlation was performed between the phenotypic characteristics of resistance and the presence of resistance genes. Cramer'sV correlation is used to measure the association between two attributes and its value varies from 0 (stating no relationship between the attributes) to 1 (stating complete association between variables). It reaches a value of 1 only when an attribute is completely determined by the other attribute.

3 | RESULTS

This study aimed to evaluate the relative frequency of exoenzymes (exo) A, U and S genes and integron classes (I, II, and III) among MDR clinical *P. aeruginosa* isolates from burn patients in Ahvaz, southwest of Iran. A total of 355 hospitalized burn cases were admitted during the study period. Among all the burn wound samples taken from 355 patients, clinical isolates of *P. aeruginosa* were identified in 145 patients by biochemical and PCR tests. Overall, 35% (52/145) of the isolates were taken from males and 64% (93/145) from female hospitalized burn patients. The patients were 35.32 years old on average while SD was 11.74 years.

3.1 | Phenotypic characteristics of the recovered isolates

In mixed cultures, *P. aeruginosa* was isolated as clear colonies on MacConkey agar (as it does not ferment lactose). *P. aeruginosa* produced colonies with a characteristic "grape-like" or "fresh-tortilla" odor on bacteriological media. A gram stain was performed, which showed Gram-negative rods. Confirmatory tests included the blue-green pigment pyocyanin production on cetrimide agar and growth at 42°C. Also, it was citrate, catalase, and oxidase positive.

Figure 1 showed randomly selected *P. aeruginosa* isolates on different culture media.

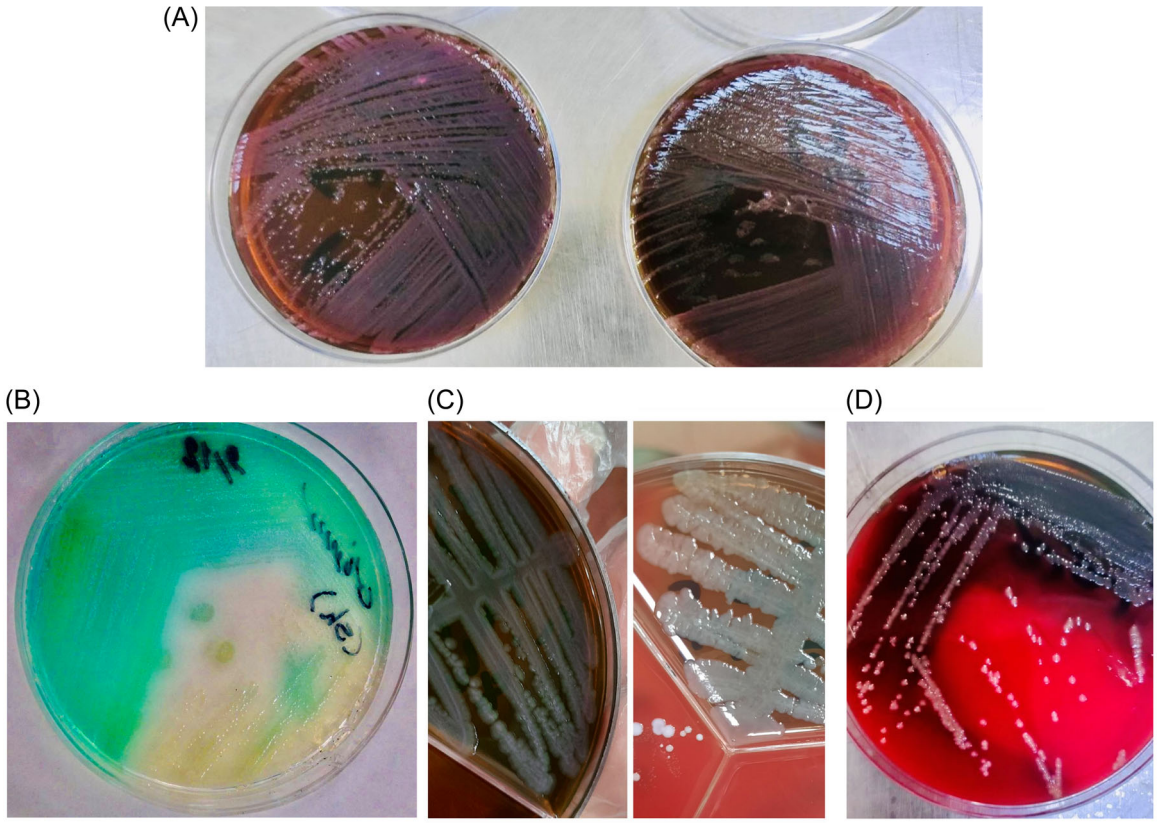

FIGURE 1 The colonial morphology of *P. aeruginosa* isolates on different culture media. (A) Red-brown colonies of *P. aeruginosa* on MacConkey agar. (B) bluish green pyocyanin pigment production on the Mueller Hinton agar. (C, D) Mucoid colony of *P. aeruginosa* on blood agar.

3.2 | Distribution of antibiotic resistance among the bacteria isolates

The maximum resistance rate among *P. aeruginosa* isolates to antibiotics tested was as follows: piperacillin 59% ($n = 86/145$), piperacillin-tazobactam 57% ($n = 83/145$), ceftazidime-avibactam 60% ($n = 87/145$), ceftolozane-tazobactam 45% ($n = 66/145$), ticarcillin-clavulanate 48% ($n = 71/145$), ceftazidime 76% ($n = 111/145$), aztreonam 76% ($n = 111/145$), doripenem 63% ($n = 92/145$), imipenem 63% ($n = 92/145$), meropenem 59% ($n = 86/145$), tobramycin 69% ($n = 101/145$), Amikacin 64% ($n = 94/145$), netilmicin 62% ($n = 90/145$), ciprofloxacin 95% ($n = 139/145$), Levofloxacin 68% ($n = 100/145$), norfloxacin 81% ($n = 118/145$), and ofloxacin 65% ($n = 95/145$). The lowest resistance rates were related to colistin (24%, 35/145), gentamicin (26%, 39/145), and gatifloxacin (29%, 43/145). A total of 100% isolates were resistant to at least one antibiotic. The MDR and XDR *P. aeruginosa* prevalence was 60% ($n = 82$) and 29% ($n = 43$) respectively. The resistant isolates had significant prevalence higher than the susceptible *P. aeruginosa* isolates. The phenotypic antibiotic resistance prevalence, diversity, and pattern of the 145 *P. aeruginosa* isolates from wounds are recorded in Tables 1 and 2.

The MAR index of the isolates revealed that all *P. aeruginosa* had greater than 0.20 index values (0.26–1) which implied that they were from high-risk environments (Table 3).

3.3 | Prevalence of exoa, exos and exou genotypes and integrons I, II, and III genes

In this study, the overall prevalence of integrons and exotoxin genes was 96.6% (140/145). The prevalence of integron classes I, II, and III in *P. aeruginosa* was 60% ($n = 87$), 7.58% ($n = 11$), and 3.44% ($n = 5$), respectively. In addition, 70(48%) of *P. aeruginosa* isolates did not harbor integron genes. The prevalence of *exoA* in *P. aeruginosa* was 55% ($n = 81$), while *exoS* and *exoU* prevalence was 55% ($n = 81$) and 56% ($n = 82$), respectively. The integron class I genes accounted for the highest frequency of genes. The coexistence of exotoxin genes is shown in Table 4.

3.4 | Correlation between antibiotic resistance and presence of genes

According to our results, *IntI* was more common in MDR and XDR *P. aeruginosa* isolates. Prevalence of MDR and XDR *P. aeruginosa* isolates in integrons I, II, and III are shown in Table 5. As shown in Tables 5, 19.31% (28/145) of *P. aeruginosa* isolates had coexistence of exotoxin genes. Besides, Table 6 shows the distribution of *exoU*, *exoS*, *exoA* in MDR and XDR *P. aeruginosa* isolates. With a statistical correlation study, it was determined that there was a significant

TABLE 3 Results of antimicrobial resistance tests by disk agar diffusion method.

Antimicrobial category	Antimicrobial agent	Resistant	Susceptible
Antipseudomonal penicillins + β-lactamase inhibitors	Piperacillin	86 (59%)	59 (40%)
B-LACTAM COMBINATION AGENTS	Piperacillin-tazobactam	83 (57%)	62 (42%)
	Ceftazidime-avibactam	87 (60%)	85 (58%)
	Ceftolozane-tazobactam	66 (45%)	100 (68%)
	Ticarcillin-clavulanate	71 (48%)	74 (51%)
Antipseudomonal cephalosporins	Ceftazidime	111 (76%)	34 (23%)
MONOBACTAMS	Aztreonam	111 (76%)	34 (23%)
CARBAPENEMS	Doripenem	92 (63%)	53 (36%)
	Imipenem	92 (63%)	53 (36%)
	Meropenem	86 (59%)	59 (40%)
AMINOGLYCOSIDES	Gentamycin	39 (26%)	106 (73%)
	Tobramycin	101 (69%)	44 (30%)
	Amikacin	94 (64%)	51 (35%)
	Netilmicin	90 (62%)	55 (37%)
FLUOROQUINOLONES	Ciprofloxacin	139 (95%)	6 (1%)
	Levofloxacin	100 (68%)	45 (31%)
	Norfloxacin	118 (81%)	27 (18%)
	Ofloxacin	95 (65%)	50 (34%)
	Gatifloxacin	43 (29%)	41 (28%)
LIPOPEPTIDES	Colistin	35 (24%)	113 (77%)

TABLE 4 Genotypic characteristic of exotoxin genes in *P. aeruginosa* isolates.

Genotype	N%	IntI n/145 (%)	IntII n/145 (%)	IntIII n/145 (%)	*P value*
exoU+ /exoS+	18 (12.41)	7 (4.82)	1 (0.68)	1 (0.68)	<0.004*
exoA+ /exoS+	19 (13.10)	5 (3.44)	2 (1.37)	2 (1.37)	<0.001*
exoA+ /exoU+	18 (12.41)	6 (4.13)	3 (2.06)	1 (0.68)	<0.001*
exoU+/exoS +/exoA+	28 (19.31)	9 (6.20)	2 (1.37)	1 (0.68)	<0.001*
exoS+	22 (15.17)	9 (6.20)	2 (1.37)	11 (7.58)	0.011
exoA+	13 (8.96)	4 (2.75)	1 (0.68)	1 (0.68)	0.266
exoU+	15 (10.34)	9 (6.20)	1 (0.68)	5 (3.44)	0.021

*It is statistically significant.

TABLE 5 The distribution of integrons in MDR and XDR *P. aeruginosa* isolates.

Antibiotic resistant	IntI n/145 (%)	IntII n/145 (%)	IntIII n/145 (%)	P value
MDR	30 (19)	7 (0.04)	1 (0.00)	<0.001*
XDR	19 (42)	2 (0.01)	1 (0.00)	<0.001*
non-XDR and MDR	30 (19)	0 (0.00)	1 (0.00)	0.210

*It is statistically significant.

TABLE 6 Distribution of *exoU*, *exoS*, *exoA* in MDR and XDR *P. aeruginosa* isolates.

Genotype	MDR	XDR	P value
exoU + /exoS + /exoA +	**25**	**3**	**<0.001***
exoA + /exoS +	**8**	**6**	**0.327**
exoA + /exoU +	**12**	**3**	**0.002***
exoS + /exoU +	**12**	**2**	**0.002***
exoS +	**16**	**6**	**<0.001***
exoA +	**6**	**1**	**0.166**
exoU +	**17**	**1**	**<0.001***

*It is statistically significant.

relationship between MDR isolates and the occurrence of the *exoU* gene (Table 7).

4 | DISCUSSION

P. aeruginosa is a nosocomial pathogen with MDR that can cause fatal infections in critically unwell individuals.[28] Colistin has recently been used as an antibiotic medication for advanced MDR P. aeruginosa infections. Some isolates (24%) in this investigation were colistin-resistant. In the absence of alternative therapies, resistance to this antibiotic can neutralize therapeutic measures.[29] Furthermore, the findings of this study revealed that MDR *P. aeruginosa* strains were disseminated throughout various clinical wards in our hospital, indicating a lack of appropriate supervision on this issue at this hospital; thus, infection control measures should be implemented to prevent the transmission of *P. aeruginosa* strains. In biomedical papers, many classifications have been used to define multidrug resistant isolates of *P. aeruginosa*. MDR was characterised in the majority of studies as acquired resistance to at least one drug in three or more antimicrobial categories, primarily aminoglycosides, anti-pseudomonal penicillins, cephalosporins, carbapenems,

TABLE 7 Results of Cramer's V correlation coefficient test.

Antibiotic resistant	Genes					
	IntI	*IntII*	*IntIII*	*exoU*	*exoS*	*exoA*
MDR	V2 = 0.133 p = 0.109	V2 = 0.070 p = 0.398	V2 = 0.161 p = 0.053	V2 = 0.225 p = 0.007	V2 = 0.104 p = 0.210	V2 = 0.128 p = 0.124
XDR	V2 = 0.108 p = 0.194	V2 = 0.044 p = 0.600	V2 = 0.056 p = 0.501	V2 = 0.141 p = 0.089	V2 = 0.076 p = 0.359	V2 = 0.148 p = 0.074

and fluoroquinolones.[20,30] Given that the samples were collected from a burn unit, the high incidence of MDR patients in the current investigation may be rationalized. The presence of such high resistance *P. aeruginosa* is not unusual in our region, since Anvarinejad et al. and Sarhangi et al. previously demonstrated a high rate of MDR among isolates from burn patients and clinical isolates from Shiraz City, respectively.[24,31]

Because detecting virulence genes in *P. aeruginosa* clinical isolates is significant,[20] the frequency of several *P. aeruginosa* virulence genes was studied in the current investigation. Most *P. aeruginosa* strains have *exoA*, *exoS*, and *exoU* genes, however the quantities of *exoA*, *exoS*, and *exoU* in isolates vary and are mutually exclusive. However, research have revealed that the frequency of cytotoxin-encoding genes vary[32,33] Perhaps because the genes producing the cytotoxins *exoS* and *exoU* vary depending on the background or site of infection in *P. aeruginosa*.[34] Furthermore, *exoU* had the highest prevalence (56%) and was shown to be lower than the number reported in Bulgaria (85.80%).[35,36] In another study in Iran, *exoU* and *exoS* had a lower rate. Fazeli et al. reported the rate of *exoS* was 67.60%.[37] It has been proposed that the infection location and length of sickness impact *P. aeruginosa* clinical isolate pathogenicity by modifying the generation of certain virulence determinants. Some anatomical regions, for example, increase the creation of *exoA* and *exoS*.[23] The prevalence of *exoS* was higher in a study conducted in Poland by Wolska et al. (75.8% vs. 55%).[38] The *exoU* appears to have been acquired by a mobile element (plasmid) incorporated into the chromosome of *P. aeruginosa*. As a result, the gene's lower frequency than other virulence genes may be attributed to this phenomena.[39] In our research, however, this rate was the same as the *exoS* prevalence (56% vs. 55%). Mitove et al.[40] observed that the prevalence of *exoS* was 62.4% in 202 cystic fibrosis (CF) and non-CF individuals, whereas the prevalence of *exoU* was 30.2%, which contradicts our findings. Surprisingly, in a French survey, the prevalence of *exoS* was significantly greater than in other investigations (94% in CF isolates vs. 80% in non-CF isolates).[41] MDR resistance was not significantly related to the virulence gene carriage rate (p = 0.124). The antibiotic resistance of (wound) isolates increased. *P. aeruginosa* wound isolates were resistant to all antibiotics in 13% of cases, demonstrating the need of creating therapeutic guidance and exerting control over nosocomial infections. In terms of antibiotic exposure pattern, colistin was shown to be the best antibiotic agent, with a 30% resistance rate. The emergence of antibiotic resistance and multidrug resistance *P. aeruginosa* isolates in clinical settings has posed a global challenge to nosocomial infection therapy.[42] Wareham et al. discovered a strong relationship between the distribution of *exoS*+ and *exoU*+ genotypes and resistance, which is consistent with our findings.[43] Feltman et al. have reported similar findings, establishing a link between the *exoS* + /*exoU*+ genotype and CF isolates.[44]

P. aeruginosa possesses a wide range of pathoadaptive features and virulence mechanisms that allow it to colonize, survive, and reproduce in a variety of settings. When *P. aeruginosa* comes into contact with a host cell, the type III secretion system (T3SS) activates, allowing it to inject released toxins (*ExoS*, *ExoY*, *ExoT*, and *ExoU*) straight into the host's cytoplasm via a syringe-like mechanism. These effector proteins have diverse roles in cytotoxicity during the bacterium's invasion and dissemination. During the infection process, various systems are activated, including the type II secretion system (T2SS), which secretes virulence agents such elastase, exotoxin A, alkaline phosphatase, and phospholipase C into the extracellular space.[45]

Moreover, *P. aeruginosa* biofilm formation can result in losing antibacterial vulnerability and increasing antibiotic concentrations in treating infections induced by these isolates. The biofilm structure can shield microorganisms against immune cells and antibiotics. Biofilm generation seems to act as a survival strategy for bacteria in case they face antibiotic agents, particularly in strains with an insufficient level of antibiotic resistance.[21] *P. aeruginosa* is resistant to several antibiotics, including aminoglycosides, quinolones, and β-lactams. In general, *P. aeruginosa*'s principal methods for resisting antibiotic assaults are classed as intrinsic, acquired, and adaptive. *P. aeruginosa*'s inherent resistance comprises poor outer membrane permeability, the development of efflux pumps that remove antibiotics from the cell, and the creation of antibiotic-inactivating enzymes. *P. aeruginosa* can gain resistance by horizontal gene transfer or mutational alterations. *P. aeruginosa*'s adaptive resistance involves the creation of a biofilm in infected individuals, which acts as a diffusion barrier, limiting antibiotic access to bacterial cells.[46]

Currently, an important feature that has been explored in the analysis of the genetic underpinnings of *P. aeruginosa* multi-resistance is the integron and the related gene cassettes. Resistance gene cassettes, which are carried by integrons on transmissible plasmids, transposons, and chromosomes, may produce antimicrobial resistance.[47] In accordance to our results, Khosravi et al. found that colistin was the most viable and favorable drug in managing nosocomial infections.[48] Fazeli et al. found a lower rate of resistance for cephems and quinolones agents.[37] Accordingly, ciprofoxacin and ceftazidime resistance rates were reported to be 63% and 63.10%, respectively. Our research revealed increased resistance to cephems and quinolones, notably ciprofoxacin and ceftazidime, showing the frequency

and relevance of integrons I and II in P. aeruginosa isolates with significant antibiotic resistance. The prevalence of integron classes I, II, and III in *P. aeruginosa* was 60%, 7.58%, and 3.44%, respectively; however, one isolate concurrently carried both integron gene classes. We also found that, *intI* prevalence in clinical isolates was in line with the majority of previous reports from 27.5% to 66%.[14,49] The high frequency of *intI* can help to acquire and disseminate antibiotic resistance genes among pathogens.[50] Given that class I integrons carry several antibiotic resistance gene cassettes encoding resistance to a wide variety of antibiotics in *P. aeruginosa*, this is not surprising. Resistance to the antibiotics was also found in additional integron-negative isolates. The acquisition of the isolates' antibiotic-resistance genes might be attributed to chromosomal-encoded enzymes or other mobile elements. Some studies in Iran found differing frequencies of the class I gene in clinical isolates of *P. aeruginosa* due to inappropriate antibiotic usage, geographical dispersion, and infection origin. Unlike our results, some have reported dissimilar frequencies of integrons II in clinical *P. aeruginosa*. Khosravi et al. showed that integrons class II was not harbored by any isolates (0%).[48] The MDR isolates proportion among integron-positive isolates was much higher than negative isolates, which shows the importance of these isolates in disseminating resistance genes among pathogens. Consequently, high resistance to antibiotics can be due to unsuitable antibiotic applications, selective pressure, etc. Our obtained results demonstrated the importance of class I and class II integrons in multiple antibiotic resistance and their relation to MDR *P. aeruginosa* isolates. Integrons help to obtain and disseminate antibiotics resistance genes among pathogens, and hence it is essential to manage the infection control policies and use antibiotics appropriately to control antibiotics resistance genes dissemination.

5 | CONCLUSION

In this study, we observed a high prevalence of MDR *P. aeruginosa* (60%). To prevent the formation of *P. aeruginosa* strains which may be MDR, an antimicrobial susceptibility test, especially MIC, should be performed before starting the treatment, and adequate supervision is required for the use of antibiotics. Regarding the high prevalence of the *intI* gene (60%) and *exo* genes and their effect on increasing the *P. aeruginosa* antibiotic resistance, the determination of positive cases and precise detection of antibiotic susceptibility patterns is strongly recommended. These results indicate the potential risk of these isolates in nosocomial infections which merit more attention. Of course, further studies are required with a larger sample size and from other regions of the country to reach a comprehensive conclusion.

AUTHOR CONTRIBUTIONS

Sousan Akrami: Conceptualization; investigation; methodology; writing—original draft; writing—review and editing. **Alireza Ekrami**: Formal analysis; investigation; visualization. **Fatemeh Jahangirimehr**: Investigation; software; supervision. **Arshid Yousefi Avarvand**: Conceptualization; formal analysis; investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data is in article. All authors have read and approved the final version of the manuscript. Arshid Yousefi Avarvand had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. Arshid Yousefi Avarvand affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted.

ETHICS STATEMENT

The authors all agree with the publication of the data in this article.

TRANSPARENCY STATEMENT

The lead author Arshid Yousefi Avarvand affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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